The molecular implications of a caspase-2-mediated site-specific tau cleavage in tauopathies

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A major focus of current experimental therapies for neurodegenerative diseases is on modulating post-translational modifications (PTMs) of the microtubule-associated protein tau. Tau is a highly soluble, neuronal protein that is comprised of four domains – the N-terminal projection domain, the proline-rich region, the microtubule-binding domain, and the C-terminal tail. As a scaffold protein, tau dynamically interacts with numerous structural and functional biomolecules, such as cytoskeleton and motor proteins, chaperones, enzymes, DNA, RNA, and lipids. Over a dozen types of PTMs, combined with alternative splicing, confer upon tau its enormous structural heterogeneity, which subserves its many (patho-) physiological functions.

Under normal conditions, the modified tau forms are actively involved in regulating a diverse set of processes, including nerve cell differentiation, neuronal morphogenesis and plasticity, neurite polarity, axon outgrowth and elongation, cargo transport along axons, synaptic plasticity, genome stability, and outgrowth of oligodendrocytes (reviewed in Arendt et al. (2016)). Upon encountering cellular stress, the carefully choreographed tau PTMs go awry, leading to the generation of toxic forms, a pathological feature that is present in a group of neurodegenerative disorders known as tauopathies (reviewed in Arendt et al. (2016)). For example, tau phosphorylation and truncation may weaken its binding to microtubules, leading to the accumulation of tau to subcellular compartments (e.g., dendritic spines and nuclei) other than axons, impairing cellular function. In this perspective, we review the impact of a caspase-2-mediated site-specific tau cleavage on synaptic and cognitive function in tauopathies, and discuss the potential of targeting caspase-2 as a therapeutic strategy against cognitive decline.

Identification of a tau cleavage product that impairs synaptic transmission: *Soluble forms of tau impair cognition in tauopathies.* Under pathophysiological conditions, tau assumes various structurally distinct forms, among which neurofibrillary tangles (NFTs) are the most extensively studied. NFTs, a pathological hallmark of at least a dozen tauopathies, including Alzheimer's disease (AD), are comprised of insoluble, intracellular, paired-helical filaments of hyperphosphorylated tau. NFTs have long been believed to drive cognitive decline in AD, because the spread of NFTs in the brain correlates with the extent of cognitive deficits. However, in experimental models, cognitive deficits can occur in the absence of NFTs, and be dissociated from NFTs. In the tau-transgenic rTg4510 mouse line, which expresses the proline-to-leucine mutation at amino acid 301 (P301L) associated with frontotemporal dementia and parkinsonism linked to chromosome 17, cognitive deficits occur before NFTs emerge, and suppressing transgenic tau expression after NFTs appear ameliorates memory impairment without reducing the NFTs (Santacruz et al., 2005). These findings implied that the memory-impairing culprits are not NFTs, and spurred the search for soluble forms of tau causing deficits in rTg4510 mice.

Δtau314, a soluble, brain-derived tau fragment, is associated with memory impairment. An exhaustive investigation of the correlation between various soluble tau species and cognitive function in rTg4510 mice led to the identification of a ~35-kDa tau fragment, whose levels correlate with the severity of impairment in a spatial reference memory test (Zhao et al., 2016). A combination of immunological techniques coupled to mass spectrometry revealed this brain-derived tau fragment to be an N-terminally-intact but C-terminally-truncated protein ending at aspartate 314 (D314) (Zhao et al., 2016), hence the name Δtau314. *In vitro* aggregation and sedimentation assays showed that Δtau314 forms Thioflavin T-reactive fibrils less readily and precipitates to a smaller extent than its full-length tau precursor (Zhao et al., 2016), likely due to near-complete truncation and elimination of the paired-helical filaments core that spans amino-acids valine 306 to phenylalanine 378 (Fitzpatrick et al., 2017).

The protease that catalyzes the cleavage of tau to form Δtau314 is caspase-2. Proteases that cleave after aspartate residues include caspases, matrix metalloproteases, and granzyme B. Based on the residues flanking D314, the strongest candidates for hydrolyzing tau to form Δtau314 are members of the caspase family. An *in vitro* cleavage assay identified caspase-2 as the sole catalyst among eight caspases expressed in human central nervous system capable of producing Δtau314 (Zhao et al., 2016).

Caspase-2-catalyzed cleavage of tau at D314 leads to synaptic dysfunction: *Caspase-2 and Δtau314 are required for tau to accumulate in dendritic spines.* While concentrated in axons, small amounts of tau also normally appear in dendritic spines, into which tau shuttles proteins that modulate excitatory post-synaptic transmission, including the tyrosine-protein kinase Fyn (Ittner and Ittner, 2018). Under pathological conditions, an excess of tau accumulates in dendritic spines, partially shutting down excitatory post-synaptic transmission (Hoover et al., 2010). In experimental models, this reduction in synaptic activity is an early pathological process that causes neurological dysfunction before apparent synaptic or neuronal degeneration occurs. When expressed in rodent primary hippocampal neurons, tau P301L accumulates abnormally in dendritic spines, whereas either rendering tau P301L resistant to caspase-2 by mutating aspartate-to-glutamate at amino acid 314 (D314E) or genetically ablating caspase-2 prevents tau P301L from accumulating in spines (Zhao et al., 2016). Thus, the earliest pathophysiological changes in synaptic function occur when tau accumulates excessively in dendritic spines, a process that depends on the generation of Δtau314 by caspase-2.

Accumulation of tau in dendritic spines is also regulated by phosphorylation. In cultured rodent neurons, wild-type human tau (tau WT) does not accumulate in dendritic spines. However, pseudo-phosphorylation by substituting glutamate for serine (S) 396 or threonine (T) 404 enables tau WT to accumulate in spines (Teravskis et al., 2019). Conversely, replacing S396 and T404 with alanines to abolish phosphorylation, prevents tau P301L from accumulating in spines (Teravskis et al., 2019). Taken together, these results indicate that the accumulation of tau in dendritic spines is regulated by both caspase-2 cleavage within the microtubule-binding domain and phosphorylation in the C-terminal tail of tau.

Phosphorylation in the proline-rich region of tau reduces excitatory post-synaptic neurotransmission. The accumulation of tau P301L within dendritic spines is associated with the internalization of functional glutamatergic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors from the post-synaptic membrane, which causes a reduction in the amplitude of miniature excitatory post-synaptic currents (mEPSCs) (Hoover et al., 2010). However, the accumulation of tau in spines *per se* is insufficient to induce synaptic dysfunction. In cultured neurons, tau WT that is pseudo-phosphorylated in the C-terminal tail accumulates in dendritic spines, but does not reduce mEPSCs unless at least one of five residues (i.e., S202, T205, T212, T217, and T231) in the proline-rich region is also pseudo-phosphorylated (Teravskis et al., 2019). How tau disrupts post-synaptic anchoring of AMAR receptors and whether synaptic function modulators (e.g., Fyn) are involved remain unclear. One possible scenario is that proline-directed S/T phosphorylation in the proline-rich region enhances the binding of tau to calcineurin, which mediates internalization of AMPA receptors by dephosphorylating the GluA1 subunit of the receptor (Miller et al., 2014).

Caspase-2-cleavage of tau induces cognitive deficits: *Caspase-2-catalyzed cleavage of tau at D314 causes cognitive deficits in tau P301L-expressing mice.* Memory impairment in rTg4510 is reversed when morpholino antisense oligonucleotides against mRNA of the murine caspase-2 (*Casp2*) gene are infused into the lateral ventricles (Zhao et al., 2016). This restoration of memory function is accompanied by approximately 35% lower levels of both caspase-2 protein and Δtau314, suggesting that caspase-2 mediates cognitive dysfunction through the processing of tau at D314. Expressing tau P301L in 2–3-month-old mice induces cognitive deficits, but expressing tau P301L D314E, which resists cleavage by caspase-2, does not (Zhao et al., 2016), providing additional support for the causative role of this site-specific cleavage event in producing cognitive abnormalities.

Of note, we have recently identified this caspase-2-mediated site-specific tau cleavage in a series of mouse lines modeling various types of tauopathies (e.g., frontotemporal dementia, AD, and Huntington's disease (HD)), and found associations with neuropathological and functional phenotypes, such as brain atrophy, premature mortality, and seizures, in addition to impaired cognition (Liu and Ashe, manuscript in preparation), supporting its broad impact on the pathogenesis of neurodegenerative disorders.

An unresolved, unexpected observation. It is puzzling that expressing Δtau314 in 2–3-month-old mice causes neither alterations in synaptic transmission nor impairments in spatial reference memory, despite its prominent accumulation in the dendritic spines (Zhao et al., 2016). Although not proven yet, it is possible that additional PTMs are required for Δtau314 *per se* to impair synaptic function, such as S/T phosphorylation in the proline-rich domain (Teravskis et al., 2019) or acetylation of lysine residues in the second microtubule-binding repeat (Tracy et al., 2016).

The impact of Δtau314 on dementia in humans: *Δtau314 levels are elevated in multiple tauopathies.* Δtau314 proteins arise from all six tau splice isoforms expressed in the central nervous system (Liu et al., 2020). Their levels are elevated in the temporal gyrus of individuals with AD or

mild cognitive impairment (Zhao et al., 2016; Liu et al., 2020) and Lewy body dementia (Smith et al., 2019), and in the prefrontal cortex and caudate nucleus of individuals with HD (Liu et al., 2019). These findings suggest a connection between Δtau314 and cognitive impairment in multiple disorders. Interestingly, levels of Δtau314 predict cognitive impairment in Lewy body dementia as effectively as the stages of Lewy body pathology (Smith et al., 2019). Given that NFTs and other forms of tau neuropathology vary markedly between brain regions, future studies on tracking relationships between Δtau314 levels in different brain structures and clinical disease progression will enhance our understanding of its role in the pathogenesis of dementing disorders.

Currently, we have not been able to detect Δtau314 reliably and reproducibly in biological fluids (e.g., cerebrospinal fluid and plasma/serum), but are developing better antibodies and protocols to overcome this shortcoming. The ability to measure Δtau314 would be invaluable for assessing Δtau314 as a molecular biomarker of synaptic dysfunction in tauopathies.

Caspase-2 as a potential therapeutic target for treating dementia: Converging evidence from studies in enzymology, structural biology, physiology, and clinical trials suggests that caspase-2 is a promising target for improving synaptic transmission in neurodegenerative conditions.

Caspase-2 has unique enzymatic and structural characteristics (reviewed in Poreba et al. (2013)). For example, caspase-2 is the only caspase with a well-defined S5 subsite. Additionally, a salt bridge between glutamate 217 and arginine 378 that is solely present in caspase-2 regulates substrate/inhibitor recognition. Further, the exclusive presence of a disulfide connection between the two small subunits is the key to maintaining the structure of the hetero-tetrameric, active enzyme. Exploiting some of these features may help in the development of a potent and selective inhibitor of caspase-2.

Casp2-knockout ($Casp2^{KO/KO}$) mice have the same median life-expectancy as wild-type mice, indicating that it is not an indispensable enzyme. However, they exhibit impaired cognitive flexibility, fear memory, synaptic plasticity, and enhanced anxiety, and experience accelerated aging of bone, muscle, and hair pigment cells. The physiological functions of caspase-2 include controlling oocyte numbers through programmed cell death, regulating osteoclast and myoblast differentiation to maintain bone and muscle cell homeostasis, promoting *de novo* lipogenesis in the liver, and regulating liver polyploidization.

A major reason that caspase-2 is an attractive therapeutic target is that its levels and activity are abnormally upregulated in multiple pathological conditions, including fatty liver diseases, osteoporosis, and various neurodegenerative diseases (reviewed in Sladky and Villunger (2020)). In neurological disorders, caspase-2 mediates neuronal damage, synaptic change, and impairment in cognitive, psychiatric, and motor function caused by several types of stress (e.g., excitotoxicity, increased reactive oxygen species, exposure to β-amyloid or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, neonatal stroke, retinal ischemia, and transgenic expression of mutant human amyloid precursor protein, huntingtin, or tau). Therefore, inhibiting caspase-2 may be beneficial in multiple neurological indications, including AD, HD, FTDP-17, Parkinson's disease, stroke, neuroblastoma, and glaucoma (reviewed in Miles et al. (2017)), provided that the level of inhibition required to improve symptoms can be achieved without dampening its normal physiological functions.

Indeed, in rats modeling optic neuropathy intravitreal injection of a small interfering RNA (siRNA) results in its local distribution in the retina, lowering caspase-2 mRNA level by ~50%, and protecting ~98% of retinal cells from death (Ahmed et al., 2011). Encouragingly, clinical trials (ClinicalTrials.gov Identifier: NCT01064505, NCT01965106) featuring intravitreal administration of QPI-1007, a caspase-2-lowering siRNA for treatment of acute non-arteritic ischemic optic neuropathy in humans, have demonstrated the safety and efficacy of engaging caspase-2, and the U.S. Food and Drug Administration has granted orphan drug designation to QPI-1007 (http://quarkpharma.com/?page_id=23).

Despite these developments, the potential of caspase-2 as a therapeutic target for cognitive disease intervention remains challenging. Although biologics such as small interfering RNAs are clearly promising, small molecules may prove more difficult to create. There is currently no caspase-2 chemical probe that can be used for target validation in pre-clinical studies; it has not been possible to develop an inhibitor with *in vitro* potency of < 100 nM and > 30-fold selectivity relative to other caspases. The chief difficulty is that the caspase-2 binding pocket is similar to the binding pockets of the other caspases, which poses the challenge of developing a small molecule that lodges securely inside the binding pocket of caspase-2 but not of the other family members.

Conclusions: Here, we discuss the effects of caspase-2-catalyzed tau cleavage at D314 on synaptic and cognitive dysfunction, the association of Δtau314 - the soluble cleavage product - with dementia, and the advantages and challenges of targeting caspase-2 for treating cognitive decline in neurodegenerative conditions. Our current understanding of the pathophysiological processes leading up caspase-2 activation, the downstream signaling of Δtau314, the diagnostic value of Δtau314, and the most efficient ways to develop of caspase-2 inhibitors is still limited. Future studies focusing on these topics will provide deeper insights into this newly identified cleavage event, and solutions for repairing synaptic transmission caused by the production of Δtau314.

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